CAZyChip : a microarray for bacterial glycoside hydrolases (GH) detection and dynamic exploration of biodiversity for plant cell wall hydrolysis.

A. Abot[1,2,3], D. Labourdette[1,2,3], L. Trouilh[1,2,3], S. Lamarre[1,2,3], G. Véronèse[1,2,3], L. Auer[1,2,3], A. Lazuka[1,2,3], G. Hernandez-Raquet[1,2,3], B. Henrissat[4], M. O'Donohue[1,2,3], C. Dumon[1,2,3] and V. Le Berre[1,2,3]

1- Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France,
2- INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France,
3- CNRS, UMR5504, F-31400 Toulouse, France
4- AFMB UMR7257 : F-13000 Marseille, France

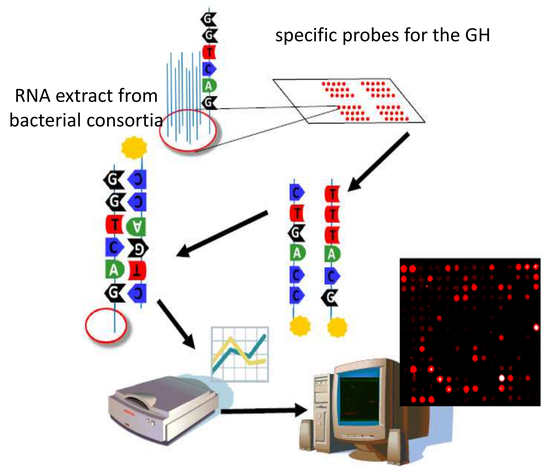
Background

Plant cell wall is a renewable and potentially sustainable source of carbon and energy. The development of biocatalysts for the deconstruction of plant cell wall polysaccharides such as cellulose and hemicelluloses is currently a major endeavor and will contribute to the development of alternative economic model, known as the **bioeconomy.**

Micro-organisms responsible for the hydrolysis of lignocellulosic polysaccharides play an important role in biotransformation of plant cell wall and produce large collections of enzymes, including **glycoside hydrolases (GH)** that are the key enzymes. GHs are classified in 133 families in the **CAZy database** (www.cazy.org).

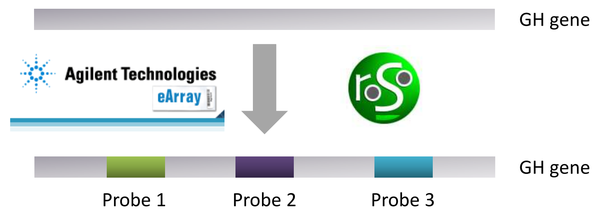
We develop a robust and generic tool, allowing the quick exploration and elucidation of functional dynamics of the enzymatic arsenal of microbial communities. This tool based on the DNA microarray principle (**CAZyChip**) allows a **rapid characterization of GH** at transcriptomic level and the characterization of plant cell wall-degrading enzyme systems that act in concert on the different polysaccharide components of lignocellulosic biomass.

DNA microarray principle



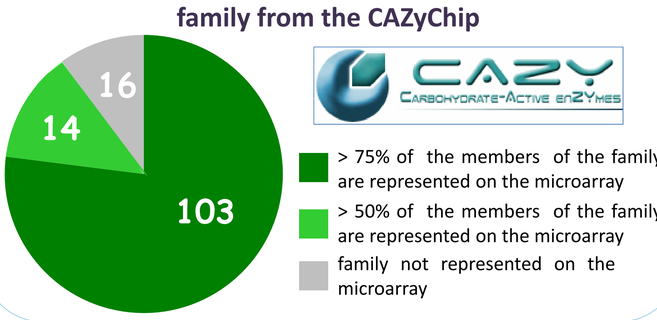
SurePrint technology (Agilent©)
Direct synthesis of probes

More than 52 000 bacterial GH are represented



Bioinformatic design using eArray and Roso softwares

Representativeness of the number of GH per family from the CAZyChip



Experimental workflow

Total RNA extraction and quality controls
- Nanodrop dosage
- BioAnalyser© profiling

cRNA synthesis, labelling and hybridation
- Agilent© technology

Scan and raw data extraction
- Feature Extraction software©

Statistical analysis
- Background correction
- Log2 data transformation
- Quantile normalization

Conclusion

The CAZyChip…

an effective, rapid and cheap screening technology

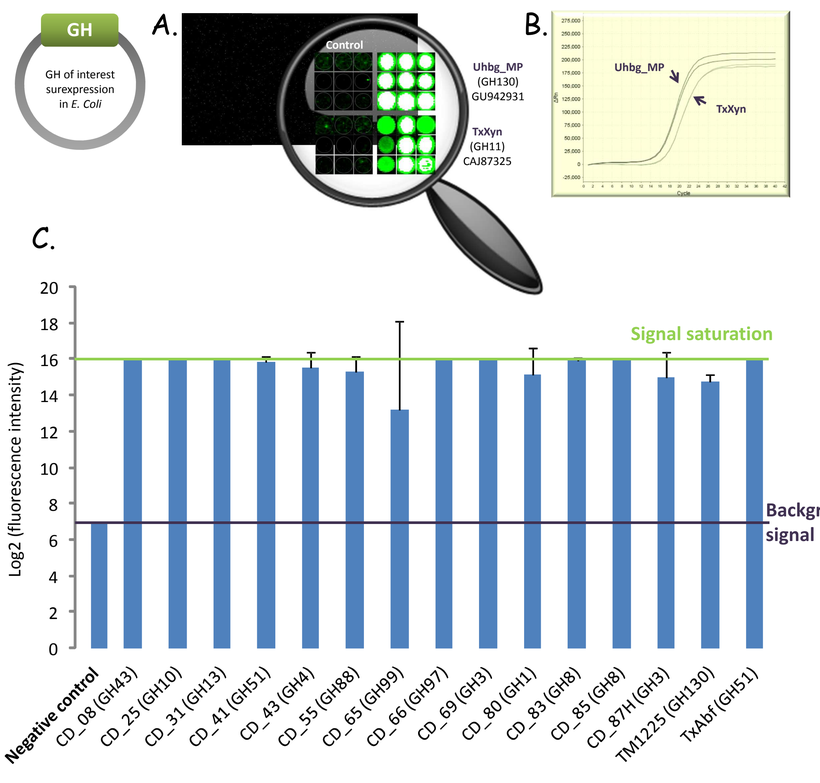
- Method to monitor the evolution of GH activity in microbial communities in this case active degrading lignocellulosic biomass.
- Study of the enzyme dynamics behind such activity via the CAZyChip will enable to identify the most active/synergistic enzymes
- Efficient tool to guide and optimize the production of enzymatic cocktails for biomass degradation
- Decision support, before using other methods such as meta-omic approaches

→ The aim of this study is consistent with the overarching challenges of the biorefinery, green chemistry, and industrial biotechnology sectors.

Validation

Cultivated organisms

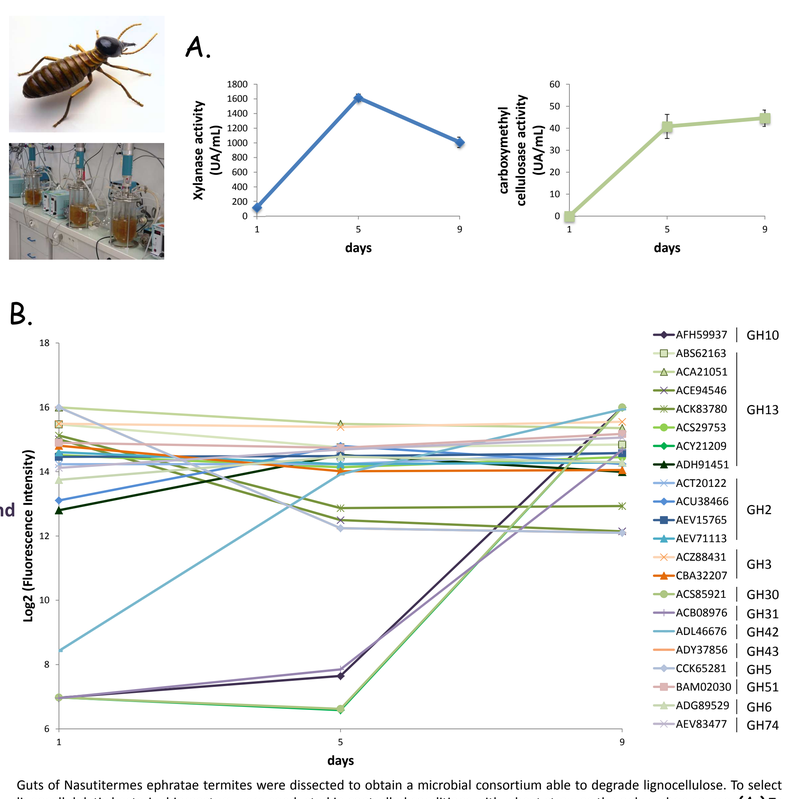
Specific detection of targeted/interesting GH



(*A.*) Example of scanned images with Innoscan© (Innopsys) on 2 genes encoding targeted/interested GH. **(*B.*)** Presence of the targeted GH transcripts is confirmed by qRT-PCR experiments. **(*C.*)** Examples of validation of probes specificity and technical reproductibility on genes encoding GH from metagenomic libraries constructed from several insects and human gut. Means ± SD are represented for the 3 probes previously designed.

Bacterial consortia screening

Detection and synergism of GH



Guts of Nasutitermes ephratae termites were dissected to obtain a microbial consortium able to degrade lignocellulose. To select lignocellulolytic bacteria, bioreactors were conducted in controlled conditions with wheat straw as the only carbon source. **(*A.*)** To validate the lignocellulolytic potential of this consortium, enzyme activities were measured at 3 different times by DNS endpoint assays on model substrates (xylan and carboxymethyl cellulose). **(*B.*)** The temporal dynamic of the transcripts at determined time points (J1, J5 and J9) is studied with the CAZyChip in order to identify the most active/synergistic enzymes. Only GH families known to degrade lignocellulose are represented.

Perspectives

Develop next microarray tool for :
- GH detection of other kingdoms (fungi, yeast…)
- Detection of other CAZymes (PL, CE, GT…)
- Suitable for other complex systems exploration and purposes such as prevention and diagnostic, in particular human intestinal microbiote.

This work was carried out with the support of GeT-Biochip platform and granted by Midi-Pyrénées region and IDEX (Paul Sabatier University).

Platform GeT- Biopuces - LISBP Bâtiment Bio5, 135, 135avenue de Rangueil - 31077 Toulouse CEDEX 4
Web : http://get.genotoul.fr
Phone : 05 61 55 94 71 / 05 61 55 96 87
E.mail : veronique.leberre@insa-toulouse.fr